REMARKS

1. Status of the Claims

Claims 27-37 are pending with entry of this amendment. Claims 27-37 were originally added by amendment October 5, 2001. Claim 27, the only independent claim, is directed to a method for obtaining a polynucleotide. Due to a typographical error, dependent claims 28-37 originally recited "the polynucleotide of claim 27." Claims 28-37 have been amended to recite "the method of claim 27," as originally intended. "Marked up" copies of the claims illustrating the amendments are attached hereto as Appendix A. As a courtesy, Appendix B provides the pending claims with entry of this amendment.

2. Election/Restrictions

Claims 28-37 were withdrawn from consideration as being directed to a non-elected invention. As described above, this was the result of a typographical error in the claims as originally presented. All of the pending claims are directed to a method of obtaining a polynucleotide. It is respectfully requested that all pending claims be considered.

3. Specification

The Examiner has objected to the title of the inventions as being insufficiently descriptive. The specification has been amended such that the title is now "Methods for Obtaining a Polynucleotide Encoding a Polypeptide Having Rubisco Activity."

3. 35 U.S.C. § 112 Rejection

Claims 10 and 16-18 were rejected under 35 U.S.C. 112, second paragraph as being indefinite. Claims 10 and 16-18 have been cancelled, rendering this rejection moot.

4. 35 U.S.C. § 103 Rejection

Claims 27 was rejected under 35 U.S.C. § 103 as allegedly unpatentable over the combination of Minshull et al., Spreitzer, and Wolter et al. The Examiner asserts that it would have been obvious to have adapted the method of Minshull et al. so as to allow for the shuffling and

screening of polynucleotide sequences that encode rubisco with desired properties as the gene for rubisco had not only been isolated and characterized and known to undergo shuffling naturally, but the gene product was of long interest in the art and the isolation of desirable mutant clones continued to be of much interest. Applicants respectfully traverse this rejection.

To establish a *prima facie* case of obviousness, there must be some suggestion or motivation to modify or combine the reference teachings. Furthermore, there must be a reasonable expectation of success, viewed in light of the prior art (MPEP 2142 and references cited therein).

As noted by the Examiner, none of the cited references explicitly teach or suggest applying the method of Minshull to the improvement of Rubisco. In the absence of an explicit suggestion or motivation to combine the reference, the Examiner asserts that the suggestion to combine the references is implicit in the references themselves and/or in the prior art. However, this assertion is effectively rebutted by the references themselves and the state of the art as generally accepted by the skilled artisan at the time the application was filed.

A. **The cited references teach away from the combination proposed by the Examiner**

It is improper to combine references where the references teach away from their combination. *In re Grasselli*, 218 USPQ 769, 779 (Fed. Cir. 1983). The Examiner asserts that Spreitzer suggests finding "desirable clones." However, a prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. See MPEP 2141.03 and references cited therein. In the section of Spreitzer cited by the Examiner (*Screening for Mutations*, page 416-18) that author is discussing screening for ***lethal*** Rubisco mutations, not Rubisco mutants having ***enhanced*** carboxylation activity as recited in the claims. The opening sentence of the *Screening for Mutations* section is "[e]ven though ***favorable Rubisco mutations cannot be selected*** directly, lethal Rubisco mutations would also be of value." The basis for Spreitzer's statement that mutants having enhanced carboxylation activity cannot be selected for directly can be found in the preceding section of the paper entitled *Selection for a Better Enzyme*, pages 415-16. In that section, Spreitzer reviews a number of unsuccessful attempts that have been made to screen for improved Rubisco mutants. In an attempt by Somerville and Ogren, 5×10^6 plants were screened without finding a single one expressing an improved Rubisco. In a similar experiment, Spreitzer et al. screened greater than 1×10^9 mutagenized *C. reinhardtii* cells without finding a single Rubisco with improved catalytic constants. Pierce et al. are described as taking a

more molecular approach, wherein their would be strong selection for a better enzyme under normal atmosphere – once again, no mutation was identified that improves Rubisco. Spreitzer concludes by stating that it will take more than a single amino acid substitution to make a better Rubisco, but if two specific amino acid substitutions are required simultaneously to make a better Rubisco, more than 1×10^{16} cells would need to be subjected to selection. He notes ruefully that there are fewer cells than this living in all the *Chlamydomonas* laboratories on the planet. Clearly, when read in its entirety Spreitzer teaches away from any attempt to screen a population of Rubisco variants for one having enhanced activity.

B. There was no reasonable expectation of successfully combining the cited references

The teaching of Spreitzer is entirely consistent with what was generally understood by those of skill in the art prior to the present invention, *i.e.*, there was no reasonable expectation that screening for an enhanced Rubisco mutant would be successful. Without this reasonable expectation of success, there can be no *prima facie* showing of obviousness.

As evidence of the general lack of reasonable expectation of success, the Examiner's attention is directed to two manuscripts that published subsequent to the priority date and which reflect the state of the art at that time. Copies of the references are submitted herewith for the Examiner's convenience.

One of these references is a review article entitled "Genetic Engineers Aim to Soup Up Crop Photosynthesis" that appeared in an edition of *Science* that published in January 1999. (Mann, C.C., (1999) *Science* 283:314-16). Near the end of page 314 the author states that the quest for a better Rubisco is a Holy Grail in plant biology, but "[d]espite more than 20 years of effort, the hopes have not yet paid off." The author of this review article does find some basis for hope in recent advances in molecular biology and the unexpected discovery of more efficient Rubisco in red algae. "In what may be the ***most ambitious genetic engineering project ever tried***, laboratories across the world are trying to improve the Rubisco in food crops by either replacing the existing enzyme with the red algae form or bolting on what could be thought of as molecular superchargers." (first partial paragraph on page 315, emphasis added). With regard to the strategy of replacing the existing enzyme with the red algae form, an expert in the field is quoted as stating that "[i]f it can be done, it would really be amazing." (first full paragraph on page 316). The leader of a team attempting that approach admits that "[i]t may not work" and that he hopes to see results in "about 10 years." (second full paragraph on page 316). The other approach cited (" bolting on molecular

superchargers") would involve introducing the C_4 cycle into C_3 plants such as rice. The author states that "the project may well be the most fundamental genetic alteration that humankind has ever tried in any organism. 'Don't hold your breath'." (top of the middle column of page 316). The author goes on to detail the many potential pitfalls to this approach that suggest a low probability of success.

The review article also discusses rational, structure-based approaches to the problem of improving Rubsico that have been attempted in recent years. (See the first paragraph of the section entitled "A better Rubisco," right column of page 315) Researchers spent years determining molecular structures of Rubsicos (the spinach and cyanobacterial forms of the enzyme are specifically discussed) and comparing the structures in an attempt to rationally engineer an improved Rubisco. These attempts were unsuccessful, dismaying many researchers and leading at least one group to disband (first sentence of paragraph bridging pages 315-316). Importantly, the article never suggests using an approach that would involve screening for an improved Rubisco variant.

More recently, a review article that appeared in *Science* just a few months ago states that "[d]ozens of research groups have [tried to alter Rubisco] to improve the efficiency of photosynthesis, but none so far have succeeded" (Gewolb, J. (2002) *Science* 295:258-59, third paragraph from the end).

In a previous Office Action, the Examiner himself apparently made reference to the low expectation of success for improving Rubisco. In the Office Action dated April 10, 2001, the Examiner states that "much interest exists in the identification of mutant genes that encode enzymes with enhanced properties. In so far as the prior art teaching the identification of such mutated Rubisco enzymes, there is very limited development in the relevant art." (page 5, section entitled The State of the Prior Art). This statement is consistent with the above-cited review articles, and suggest that the Examiner is aware of the fact that many have attempted to improve Rubisco using a variety of approaches, but prior to this invention there has been little or no success.

In summary, the record clearly supports Applicants' position that in this case there was no reasonable expectation that the cited references could be combined successfully. Spreitzer teaches that previous attempts at screening for improved Rubisco variants have been unsuccessful and postulates that further screening-based approaches would likely also fail. Recent review articles, published well after the references cited by the Examiner, do not even mention a screening approach to identifying an improved Rubsico, reflecting the view of those in the field that such attempts would

likely be futile. Instead, the 1999 *Science* article discusses rational design approaches, the introduction of cyanobacterial enzyme into plants, and the introduction of a C_4 pathway into a C_3 plant. The rational design approach has thus far proven futile, while the success of the other two proposed approaches is acknowledged to be a long shot. Surely, with the high interest in generating a better Rubsico and the lack of any truly promising strategy to achieve this result, someone of skill in the art would have at least suggested combining the cited references if they had viewed the combination as being likely to succeed.

3. The test for obviousness is not "obvious to try"

It is clearly the case that "obvious to try" is not the standard under Section 103 (MPEP 2145(X)(B) and references cited therein). Regardless of whether one of skill in the art might have found it obvious to try to apply the general teachings of Minshull to the specific problem of improving Rubisco, absent a reasonable expectation of success there would have been insufficient motivation to combine the references.

4. The Wolter reference is essentially irrelevant

The Examiner states that Wolter et al. "disclose the shuffling of rubisco gene [sic] during evolution." Wolter et al. analyzed the sequences of known Rubisco genes and "postulate that the structure of small subunit genes of $RbuP_2$ is the result of two counteracting processes working sequentially during evolution. First, introns were introduced before or during exon shuffling, adding new domains for new functions. Later, these introns were lost stepwise, leading to a more streamlined gene structure." (quoting the final paragraph). It is not the shuffling of exons that is noteworthy, but rather the introduction and stepwise loss of introns during the process. One of skill in the art will recognize that "exon shuffling" is a general mechanism thought to occur during evolution, *i.e.*, it is not something specific or noteworthy with regard to Rubsico. The skilled artisan would regard the teaching of Wolter as essentially irrelevant with respect to the obviousness of the claimed invention.

5. Claims 29-37

Due to the typographical error noted above, the Examiner did not previously consider claims 29-37. However, all of these claims depend upon claim 28, and therefore cannot be obvious if the base claim is nonobvious. Therefore, consideration of these claims should not require a new search to be done, and it is respectfully requested that these claims be considered and found allowable along with the Claim 28.

CONCLUSION

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 298-5884.

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Respectfully submitted,

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APPENDIX A

"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE CLAIMS OF 09/437,726 WITH ENTRY OF THIS AMENDMENT

28. (amended) The [polynucleotide] method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a higher carboxylation specificity factor than proteins encoded by the plurality of polynucleotide species.

29. (amended) The [polynucleotide] method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a velocity of carboxylation that is greater than that of proteins encoded by the plurality of polynucleotide species.

30. (amended) The [polynucleotide] method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a velocity of oxygenation that is less than that of proteins encoded by the plurality of polynucleotide species.

31. (amended) The [polynucleotide] method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a Km for CO_2 that is less than that of proteins encoded by the plurality of polynucleotide species.

32. (amended) The [polynucleotide] method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a Km for O_2 that is greater than that of proteins encoded by the plurality of polynucleotide species.

33. (amended) The [polynucleotide] method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I L subunit.

34. (amended) The [polynucleotide] method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I S subunit.

35. (amended) The [polynucleotide] method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form II subunit.

36. (amended) The [polynucleotide] method of claim 27 further comprising a selectable marker gene which affords a means of selection when expressed in chloroplasts.

37. (amended) The [polynucleotide] method of claim 36, wherein the sequence encoding a protein having Rubisco carboxylation activity and the selectable marker gene are flanked by an

upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and a downstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination.